APPENDIX III

MARKED-UP VERSION OF REPLACEMENT PARAGRAPHS IN THE SPECIFICATION PURSUANT TO 37 C.F.R. § 1.21 (b)(1)(iii)

Figure 3 schematically shows the vector maps, including restriction endonuclease recognition sites, of the protein expression vectors pQE-30, pQE-31, and pQE-32. The nucleotide sequence for the polylinker region of pQE-30 is provided in SEQ ID NO: 22. The nucleotide sequence for the polylinker region of pQE-31 is provided in SEQ ID NO: 23. The nucleotide sequence for the polylinker region of pQE-32 is provided in SEQ ID NO: 24.

To confirm that the *sqdX* gene in the cyanobacteria *Synechococcus* encodes functionally homologous proteins, the *sqdX* open reading frame of *Synechococcus* was inserted behind the *tac* promoter in the mobilizable broad host range plasmid pRL59EH (Black *et al.*, "Analysis of a Het- mutation in Anabaena sp. PCC7120 implicates a secondary metabolite in the regulation of heterocyst spacing," *J. Bacteriol.*, 174: 2282-2292[2352-2360] (1994)), and transferred the constructs by conjugation into *Synechococcus* mutant 7942ΔsqdX as described in Wolk *et al.*, "Construction of shuttle vectors capable of conjugative transfer from *Escherichia coli* to nitrogen-fixing filamentous cyanobacteria," *Proc. Natl. Acad. Sci. USA*, 81: 1561-565 (1984). Sequences 5' of the presumed ATG up to the first in-frame stop codon (position 2385912-2387168 of the genome sequence) were included. The *sqdX* gene of *Synechococcus* was PCR-cloned from the plasmid pSYB using the primers 5'-AAG GAT CCT GCG CTA AAG TCG CAC TC-3' (SEQ ID NO: 21) and 5'-ATA AGC TTC GAG CTC AGG CCG CT-3' (SEQ ID NO: 13) into the *Hin*d III/*Bam*H I sites of pRL59EH. An Ω cassette from the plasmid pHP45Ω (as described in Prentki, P. and Krisch, H.M., "In vitro insertional mutagenesis with a selectable DNA fragment," *Gene*, 29: 303-313 (1984)) conferring spectinomycin and streptomycin resistance was inserted into the *Hin*d III sites of these plasmids to provide a suitable selectable marker. The resulting plasmid containing *sqdX* of *Synechococcus* was designated pSQDX7942. Exconjugants were selected on BG11 medium containing 25 μg/ml kanamycin, 10 μg/ml spectinomycin, and 1 μg/ml streptomycin and were analyzed by DNA/DNA hybridization to confirm the presence of the proper plasmid construct. The insertion of the *sqdX* construct restored the sulfolipid biosynthetic activity in

the *Synechococcus* mutant 7942ΔsqdX as shown by TLC lipid analysis. Based on the observed genetic complementation, it is concluded that the cyanobacterial *sqdX* gene encodes a protein involved in sulfolipid biosynthesis.